Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2016
Biomedical Engineering

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Digestion

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1 Digestion

Estimated bench time: 30 minutes
Estimated total time: 5 hours
Purpose: Giving sticky ends to the vector and the insert(s) so that they can be ligated.
It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- 10X NEB Cut-Smart buffer
- Autoclaved H_2O
- Autoclaved PCR tubes
- Bucket with ice
- Insert(s) which is/are to be digested (pcr amplification product)
- Pipettes and tips
- Thermal cycler
- Vector which is to be digested
- Restriction enzyme BamHI-HF
- Restriction enzyme HindIII-HF

1.2 Setup & Protocol

Properties of the restriction enzymes can be found at www.neb.com

- Please check the stock concentration of the restriction enzyme you use
- Use PCR tubes. (Evt. distribute the digestion mixtures over >1 tube)
- Add all components, end with restriction enzymes (transport in cold block)
- mix well

Reaction mixture insert

Component	Quantity/mass/final concentration	Volume (µl)
DNA (PCR product ng/uL)	PCR product: ~3 ug	
10x NEB CutSmart buffer	1x	5
Restriction enzyme BamHI-HF (20 U/uL stock)	10 U	1
Restriction enzyme HindIII-HF (20 U/uL stock)	10 U	1
H20		
Total		50

Reaction mixture vector		
Component	**Quantity/mass/final concentration**	**Volume (µl)**
DNA pET28a (vector …. ng/uL)	Vector: 5 ug	
10x NEB CutSmart buffer	1x	5
Restriction enzyme BamHI-HF (20 U/uL stock)	20 U	1
Restriction enzyme HindIII-HF (20 U/uL stock)	20 U	1
H_2O		
total		50

- Start PCR machine using program shown below

Step	Temp (°C)	Time (min)
Incubation	37	240 (4 hours)
Cooling	4	hold